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The emerging therapeutic potential of sirtuin-interacting drugs: from cell death to lifespan extension

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Acetylation of chromatin-interacting proteins is central to the epigenetic regulation of genome architecture and gene expression. Chemicals that modulate the acetylation of nuclear proteins have proved instrumental in experimental models of several human diseases. Sirtuins represent a new class of evolutionary conserved histone deacetylases, originally identified in yeast, that have emerging pathogenetic roles in cancer, diabetes, muscle differentiation, heart failure, neurodegeneration and aging. In this article, we focus on sirtuins and provide an appraisal of current compounds that either activate or inhibit sirtuin activity, highlighting their therapeutic potential for the treatment of human diseases.

Since the pioneering work of the first cell biologists, who divided nuclear content into eu- and hetero-chromatin, research aimed at identifying the molecular mechanisms that underlie the homeostatic control of chromatin architecture and readability of the genetic code has progressed considerably. During the past decade, it has been well appreciated that genetic information is made available to transcription as a result of highly dynamic epigenetic modifications [1,2]. Among other processes, the acetylation of histone proteins by the concerted action of acetyltransferases and class I and II histone deacetylases (HDACs) is currently envisaged as central to epigenetics [3]. Because of the emerging pathogenetic role of histone deacetylation in human disorders, a great deal of effort has been devoted to develop chemicals that modulate HDAC activity [4,5].

In this review, we focus on sirtuins (Sir proteins), which comprise the unique class III HDACs that originally were shown to be involved in gene silencing in yeast. Following intense research, it is now clear that sirtuins are phylogenetically conserved from bacteria to humans, and regulate cell functions beyond silencing [6–10]. Indeed, recent research indicates that sirtuins have pathophysiological relevance to cancer, obesity, muscle differentiation, inflammation and neurodegeneration. In addition, experimental evidence unequivocally shows that sirtuin activity extends the lifespan of several organisms [6,8–10]. These findings have prompted researchers to modulate sirtuins pharmacologically by developing positive and negative regulators of their enzymatic activity. In this article, we provide a review of these chemicals, summarizing their pharmacodynamic properties and highlighting their therapeutic potential.

Historical perspective: Sir proteins and silencing of yeast chromatin

Sir1–4 (silent information regulator 1–4) proteins were first shown to be involved in silencing at cell-mating type loci and telomeres in yeast. Subsequent studies showed that Sir2 also participates in the silencing and suppression of recombination at yeast ribosomal DNA (rDNA). Later, additional Sir2 homologues (Hst1–4) that were also involved in silencing were identified [6–10].

Yeast sirtuins are deacetylases that are involved in a peculiar silencing process. Following the recruitment of the Sir complex (Sir2–4) onto the silencer, Sir2-dependent histone deacetylation on flanking nucleosomes prompts the binding of another Sir complex and the spreading of deacetylation. This results in a compact chromatin structure that is refractory to transcription and enwrapped by Sir proteins and other heterochromatin components [6,9]. At rDNA, Sir2, as part of a multiprotein complex, binds RNA polymerase I in such a way that Sir2-dependent chromatin modifications are coupled closely to rDNA transcription [9]. Importantly, ongoing Sir2 activity is necessary for the maintenance of silencing [11–14], and, indeed, hyperacetylation at cell-mating type loci, telomeres and rDNA occurs in yeast bearing a *sir2* deletion [15].

New clues: evolutionary conserved, NAD-dependent chromatin remodelling enzymes

Numerous Sir2 homologues have been identified in different organisms [8,9] (Table 1). Phylogenetic analysis of conserved core sequences in archeans, bacteria, yeasts, plants, protozoans and metazoans reveals that sirtuins can be grouped into five different classes (designated I–IV and U) and are present in all eukaryotes, suggesting ancestral biological functions [16,17]. SIRT1 is the human orthologue of yeast Sir2 and is the best-characterized member among mammalian sirtuins. SIRT1 is a nuclear protein that is endowed with deacetylase activity [12,18,19] and interacts with several transcription-regulating factors (see later). Of note, *Sirt1*$^{-/-}$ mice exhibit development and reproduction abnormalities but no apparent alterations in telomere length or gene

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Table 1. Sirtuin deacetylases[a–c]

	Intracellular localization	Deacetylating activity	Function or target	Refs
Yeast				
Sir1	Nucleus	No	Gene silencing	[7]
Sir2	Nucleus	Yes	Gene silencing, lifespan prolongation	[7–9]
Sir3	Nucleus	No	Gene silencing, lifespan prolongation	[7]
Sir4	Nucleus	No	Gene silencing, lifespan prolongation	[7]
Hst1	Nucleus	Yes	Gene silencing	[8–10,17]
Hst2	Cytoplasm	Yes	Gene silencing	[8–10,17]
Hst3	Nucleus	Yes	Gene silencing, radiation resistance	[8–10,17]
Hst4	Nucleus	Yes	Gene silencing, radiation resistance	[8–10,17]
C. elegans				
Sir2.1	Nucleus and cytoplasm	Unknown	Lifespan prolongation	[42]
Drosophila				
dSir2	Nucleus and cytoplasm	Yes	Lifespan prolongation	[45]
Mouse				
SIRT1	Nucleus	Yes	Embryonic development	[8,20,21]
Human				
SIRT1	Nucleus	Yes	Deacetylation of p53, Ku70, FOXO3, NF-κB and MyoD	[16,19, 57–59, 60–67]
SIRT2	Nucleus and cytoplasm	Yes	Deacetylation of tubulin	[16,23]
SIRT3	Mitochondria	Yes	Unknown	[16,24,25]
SIRT4	Unknown	No	Unknown	[8,16]
SIRT5	Unknown	Yes	Unknown	[8,16]
SIRT6	Unknown	No	Unknown	[8]
SIRT7	Unknown	No	Unknown	[8]

[a]Abbreviation: *C. elegans, Caenorhabditis elegans.*
[b]All proteins listed are homologues of yeast Sir2.
[c]The intracellular localization and deacetylase activity of sirtuins so far identified is shown.

silencing [20–22]. Mammalian SIRT2 is a cytoplasmic and nuclear deacetylase that is involved in mitosis and can target histones *in vitro* and tubulin *in vivo* [23]. Mammalian SIRT3 can deacetylate histones but is localized curiously in the mitochondrial matrix [24,25], a cell compartment that typically lacks histones. Much less is known about mammalian SIRT4–7, although they are expressed widely in human cells [8,16].

A real breakthrough in basic biochemistry and epigenetics occurred when sirtuins were identified as unprecedented NAD-dependent HDACs [18,26,27]. Sirtuins catalyze a unique deacetylation reaction that consists of the hydrolysis of the glycosidic bond of NAD between nicotinamide and ADP-ribose [28] and transfer of the acetyl group that is bound to proteins to ADP-ribose to form a new metabolite: 2′-acetyl-ADP-ribose. The latter in turn equilibrates with the regioisomer 3′-acetyl-ADP-ribose through spontaneous intramolecular transesterification [29–32] (Figure 1). With respect to the catalytic mechanism of NAD-dependent deacetylation [30,33–36], sirtuins use the energy of binding to the acetylated peptide to twist the nicotinamide ring of NAD and activate the glycosidic bond between nicotinamide and ADP-ribose. This facilitates breakage of the bond, with the release of nicotinamide and the formation of an O-alkylamidate intermediate. Charge destabilization in the enzyme–intermediate complex provides the energy that is necessary to drive the reaction forward with the release of the deacetylated substrate and 2′-acetyl-ADP-ribose. Impaired functioning at the step of O-alkylamidate formation can reverse catalysis with binding of nicotinamide to ADP-ribose and NAD re-synthesis [35,37]. This 'NAD–nicotinamide exchange reaction' or 'transglycosidation' underlies the inhibition of sirtuins by nicotinamide [12,28,36,38,39] (Figure 1). Overall, structural and mechanistic insights into NAD-dependent deacetylation can provide important information for the development of selective and powerful sirtuin inhibitors.

Sirtuins and the genetic control of aging: fungi, worms, fruit flies and more?

In 1995, Guarente and colleagues reported that the lifespan of yeast is extended by >30% in the strain mutant Sir4–42, which lacks the C-terminal domain of Sir4 [40]. Importantly, concomitant mutation of *sir2* or *sir3* suppressed lifespan extension in Sir4–42 mutants [40]. Additional work from the same group demonstrated that deletion of *sir3* or *sir4* in yeast causes loss of silencing and 20% reduction in lifespan [41]. Similarly, *sir2* deletion increases rDNA recombination and shortens lifespan by 50%, whereas *sir2* overexpression prolongs lifespan by 30%, compared with wild-type counterparts [41]. These findings, together with evidence that mutations that inactivate Sir2 accelerate senescence [18], suggest that Sir2-dependent deacetylation is a limiting factor of yeast aging. Importantly, an additional copy of *sir2.1*, the most related *sir2* homologue in *Caenorhabditis elegans*, also extends the lifespan of *C. elegans* by 50%, compared with wild type [42].

Evidence that sirtuins regulate lifespan is also derived from studies on calorie restriction [CR (a 30–60% reduction in calorie intake with respect to *ad libitum* levels)], which is the most effective strategy for extending the lifespan of different organisms, including mammals [43]. Of note, lifespan extension of a yeast strain with increased longevity because of impaired sensitivity to glucose (a condition that mimics CR) is dependent on Sir2 expression and NAD synthesis [44]. Similarly, CR prolongs lifespan in *C. elegans* and *Drosophila melanogaster* in a Sir2-dependent manner [45]. Together, these findings suggest that NAD-dependent deacetylation by Sir2 slows aging in yeast and adult metazoans. Whether this family of deacetylases also determines longevity of higher eukaryotes is unknown. Nevertheless, the fact that sirtuin activity participates in lifespan extension by CR and depends on the availability of NAD, a molecule that is central to energy metabolism, prompts the exciting hypothesis that metabolism regulates longevity at least in part through Sir2-like deacetylases [46,47].

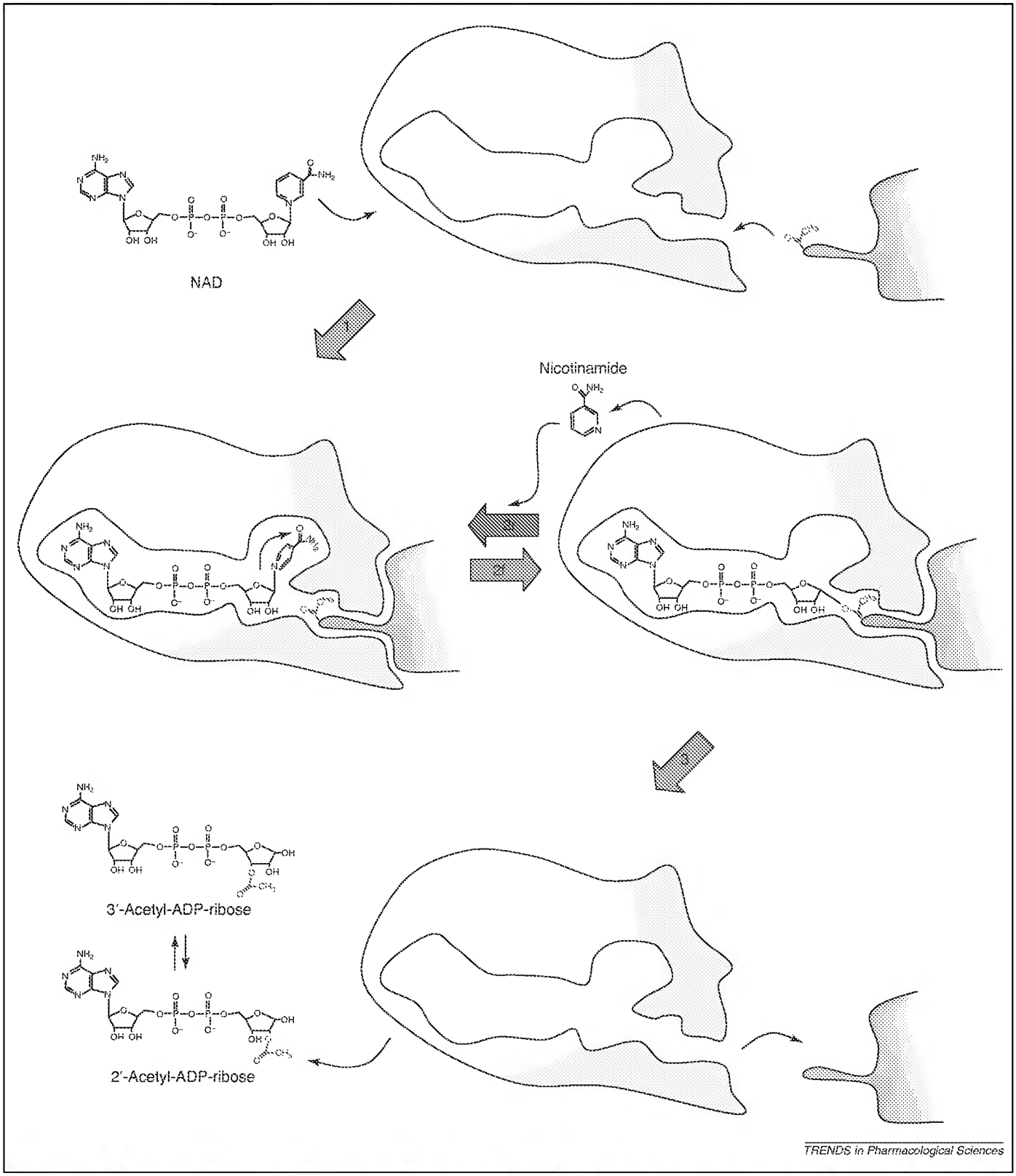

Figure 1. Sirtuin-operated NAD-dependent deacetylation. Sirtuins (yellow) bind acetylated proteins (blue) and NAD. Sirtuins use the energy of binding to the acetylated protein to twist the glycosidic bond between nicotinamide and ADP-ribose, thereby leading to a destabilized NAD conformation (Step 1). Following hydrolysis of the glycosidic bond, nicotinamide is released whereas ADP-ribose binds the acetyl-peptide with the formation of an O-alkylamidate intermediate (Step 2f). The enzyme–intermediate complex eventually releases 2′-acetyl-ADP-ribose and the deacetylated proteins (Step 3). 2′-Acetyl-ADP-ribose in turn spontaneously equilibrates with the regioisomer 3′-acetyl-ADP-ribose through transesterification. Once released, nicotinamide can re-bind the O-alkylamidate intermediate driving the reverse reaction, leading to NAD re-synthesis (Step 2r). See main text for further details.

An intriguing NAD connection

Several findings show that NAD metabolism (Figure 2) regulates sirtuin functioning [46]. For example, mutation of *npt1*, the gene encoding yeast nicotinate phosphoribosyl transferase, which participates in NAD re-synthesis, reduces the concentration of intracellular NAD by a factor of 2.5, impairs silencing at telomeres and rDNA and abolishes Sir2-dependent yeast lifespan extension [27,44,48]. Opposite effects on silencing and longevity are induced by *npt1* overexpression [49]. Similarly, additional copies of the genes involved in the NAD rescue pathway (Figure 2), *pnc1*, *nma1* and *nma2*, favours telomeric and rDNA silencing in yeast, suggesting that increased expression of these genes leads to more-efficient sirtuin activity [49]. In addition, overexpression of genes involved in mitochondrial respiration prompts Sir2-dependent yeast lifespan extension [50], probably because of increased mitochondrial re-oxidation of NADH into NAD [46]. Remarkably, an increased NAD:NADH ratio inhibits the differentiation of mouse skeletal muscle cells by promoting SIRT1 activity [19], and augmented availability of NAD in mouse neurons and fibroblasts promotes SIRT1 activity [51,52]. Together, these data suggest that in both yeast and mammals sirtuins link the redox state of the cell to cell functioning by sensing NAD concentrations.

Recent studies, however, challenge this interpretation. For example, Sir2 and SIRT1 activities are not significantly affected by the NAD:NADH ratio in an *in vitro* deacetylation assay [38]. Similarly, lifespan prolongation that is dependent on CR or *npt1* overexpression does not correlate with increased NAD levels or the NAD:NADH ratio [38,49]. Finally, yeast cells that possess deletion of the NAD rescue pathway gene *pcn1* have a silencing defect that is not paralleled by changes in NAD content [48]. Clues from work by Sinclair and colleagues might explain these apparent inconsistencies. These authors report that overexpression of *pnc1* in yeast is necessary and sufficient for CR to induce Sir2-dependent extension of lifespan by 70% [13]. Knowledge that *pnc1* encodes the enzyme that converts nicotinamide into nicotinic acid suggests that reduction of the intracellular nicotinamide content promotes sirtuin activity. This hypothesis is

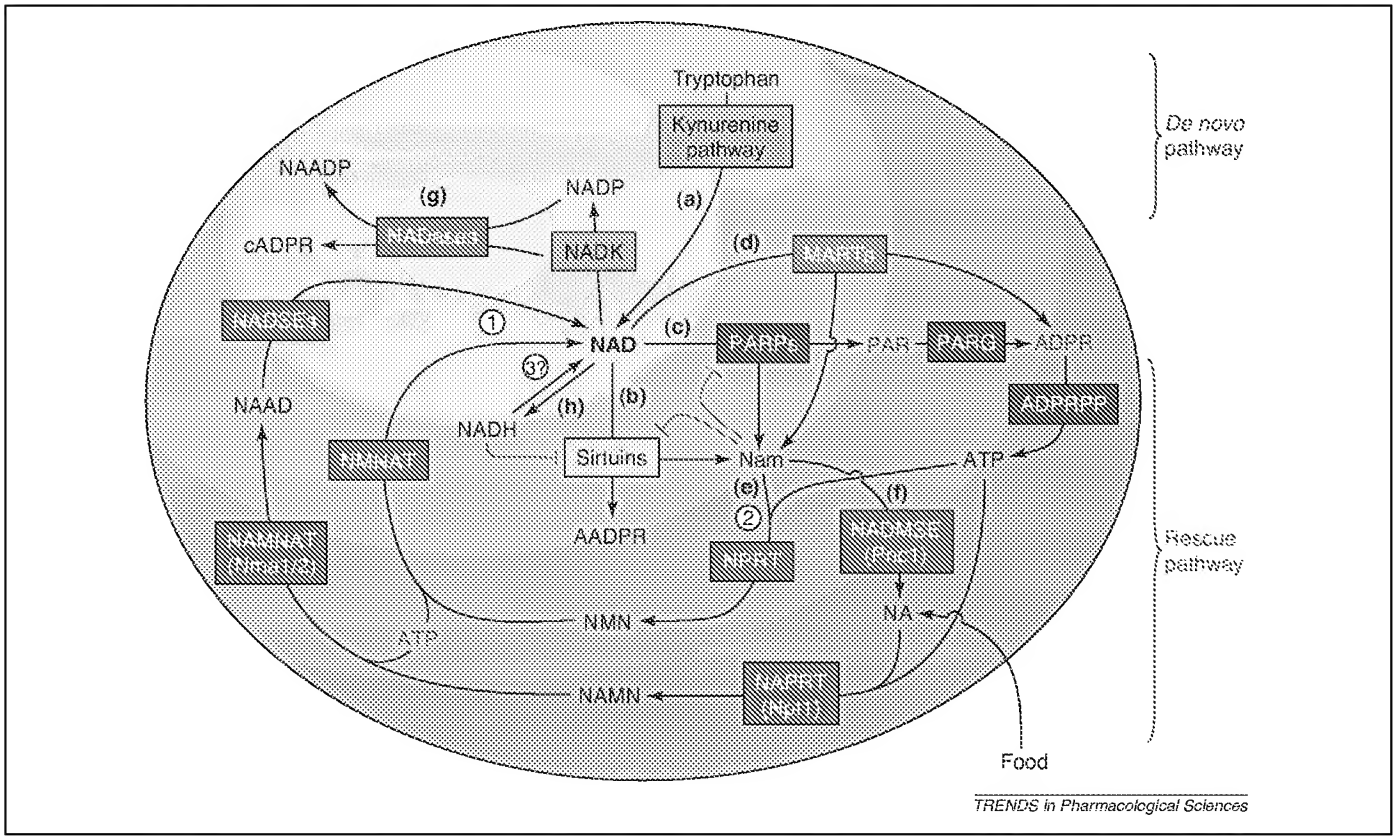

Figure 2. NAD metabolic pathways. **(a)** In mammalian cells, ~90% of free tryptophan is metabolized through the kynurenine pathway, leading to the *de novo* synthesis of NAD. NAD can be hydrolyzed into nicotinamide (Nam) and acetyl-ADP-ribose (AADPR) by sirtuins **(b)** or into nicotinamide and poly(ADP-ribose) (PAR) by poly(ADP-ribose) polymerases (PARPs) **(c)**. Note that nicotinamide inhibits both sirtuins and PARPs. PAR undergoes hydrolysis by poly(ADP-ribose) glycohydrolase (PARG), with ensuing formation of ADP-ribose (ADPR), which, in turn, can be transformed into ATP by ADP-ribose pyrophosphorylase (ADPRPP). **(d)** Mono (ADP-ribose) transferases (MARTs) also convert NAD into nicotinamide and ADP-ribose. **(e)** NAD re-synthesis through the rescue pathway starts from nicotinamide, which is first converted into nicotinamide mononucleotide (NMN) and then into NAD by the ATP-consuming enzymes nicotinamide phosphoribosyl transferase (NPRT) and nicotinamide mononucleotide adenylyl transferase (NMNAT), respectively. **(f)** Nicotinic acid (NA) can be formed by nicotinamide deamidase (NADMSE) in non-mammalian cells, whereas in humans its origin is mainly nutritional [56]. In mammalian cells, however, nicotinic acid can be converted into nicotinic acid mononucleotide (NANM) and nicotinate adenine dinucleotide [NAAD (also called deamido-NAD)] by the concerted actions of nicotinic acid phosphoribosyl transferase (NAPRT) and nicotinic acid mononucleotide adenylyl transferase (NAMNAT), respectively. Nicotinate adenine dinucleotide is directly transformed into NAD by ammonia-dependent or glutamine-hydrolyzing NAD syntheses (NADSEs). **(g)** NAD and NAD phosphate (NADP), synthesized by NAD kinase (NADK), are respectively transformed into the intracellular Ca^{2+} mobilizers cyclic-ADP-ribose (cADPR) and nicotinate adenine dinucleotide phosphate (NAADP) by the same enzymes: NAD glycohydrolases [NADases (also known as ADP-ribosyl cyclases or NAD transglycosidases)]. **(h)** Redox equilibrium between NAD and NADH, together with the possibility that the latter can inhibit sirtuin, is shown. Circled numbers indicate the three proposed mechanisms by which NAD metabolism boosts sirtuin activity: (1) increased NAD re-synthesis; (2) nicotinamide catabolism; and (3?) possible NADH oxidation. Nomenclature for the yeast enzymes of the NAD rescue pathway are indicated in brackets.

strengthened by two lines of evidence. First, physiological concentrations of nicotinamide efficiently inhibit Sir2 and SIRT1 *in vitro* [12,28,38]. Second, conversion of nicotinamide into methyl-nicotinamide enhances silencing and increases yeast lifespan [13]. Strikingly, stimuli known to increase lifespan in yeast, such as glucose or amino acid restriction in addition to osmotic shock or heat stress, increase the expression of the nicotinamide catabolizing enzyme Pcn1 [13]. Finally, two reports demonstrate that NADH is an efficient [53] or weak [39] sirtuin inhibitor and that reduction of NADH levels increases yeast lifespan [53]. In summary, NAD metabolism could promote sirtuin activity by at least three different means: increased NAD re-synthesis, augmented nicotinamide utilization, or re-oxidation of NADH (Figure 2).

It is also conceivable that sirtuin activity is impaired in conditions of NAD shortage. (Hyper)activity of NAD kinase, NAD glycohydrolase and NAD nucleosidase in addition to mono- and poly-(ADP-ribose) transferases might therefore reduce sirtuin functioning. In this regard, it is well appreciated that poly(ADP-ribose) polymerase 1 (PARP-1) can drastically deplete intracellular NAD pools following DNA damage. Curiously, akin to SIRT1, PARP-1 is inhibited by its by-product nicotinamide (Figure 2) and participates in chromatin remodelling [54]. On this basis, it is tempting to speculate a link between NAD signalling, sirtuins and poly(ADP-ribosyl)ation [55,56]. For example, impairment of SIRT1 activity because of PARP-1-dependent NAD shortage might promote p53, FOXO and Bax activities (see later), with sensitization to apoptosis. This hypothesis is in line with the active role of PARP-1 in apoptotic cell death [54].

Overall, sirtuins appear unprecedented 'NAD sensors' that link energetic metabolism to gene expression. As pointed out by Grozinger and Schreiber [5], the 1:1 stoichiometry between NAD and sirtuin-operated deacetylation warrants tight dependence and sensitivity of the deacetylases to cellular NAD content. This also suggests that pharmacological modulation of NAD metabolism is a suitable strategy to regulate sirtuin functioning in mammalian cells.

Non-histone targets

Mounting evidence demonstrates that sirtuins also target a substantial number of non-histone proteins. For example, SIRT1 deacetylates the TATA box-binding protein $TAF_{(I)}68$, thereby repressing activation of RNA polymerase I [57]. SIRT1 also targets CTIP2 (COUP-TF interacting protein 2) and enhances CTIP2-dependent silencing [58]. Similarly, sirtuin-dependent deacetylation promotes activity of the BCL6 (B-cell lymphoma 6) transcriptional repressor [59]. It is well known that acetylation of p53 promotes protein activation. Importantly, SIRT1 deacetylates p53 and protects against p53-dependent apoptosis [60,61]. Work from Kouzarides' group also shows that deacetylation of p53 by SIRT1 within pro-myelocytic leukemia protein nuclear bodies antagonizes p53 and cellular senescence [62]. Conversely, dominant-negative SIRT1 promotes p53-dependent apoptosis and enhances radiosensitivity in human cells [61]. In agreement with these findings, *Sirt1*$^{-/-}$ mice display p53 hyperacetylation and increased sensitivity to radiation-induced apoptosis [22].

Forkhead transcription factors of the FOXO subfamily are transactivators involved in growth control, differentiation and apoptosis. SIRT1 targets FOXO3a and FOXO4 and represses their transcriptional activity in a deacetylation-dependent manner. Consistently, SIRT1 reduces FOXO4-triggered apoptosis, and the expression levels of FOXO-activated genes are higher in *Sirt1*$^{-/-}$ mice [14]. Additional work shows that SIRT1-dependent FOXO3 deacetylation reduces the expression of pro-apoptotic genes and the ensuing cell death, whereas it favours cell cycle arrest and the expression of genes involved in stress resistance in eukaryotic cells [63]. In keeping with this, deacetylation of FOXO1 by SIRT1 promotes the expression of $p27^{kip1}$ and manganese superoxide dismutase (MnSOD) [64]. Together, these data suggest that SIRT1 regulates FOXO transactivation in a gene-specific manner [65].

Interestingly, SIRT1 deacetylates Lys310 of the p65 nuclear factor κB (NF-κB) subunit, thereby impairing NF-κB-driven transactivation of the pro-survival genes *Bcl-XL*, *TRAF2* and *cIAP2*, with consequent sensitization to apoptosis in eukaryotic cells [66]. SIRT1 also interacts with and represses peroxisome proliferator-activated receptor γ (PPAR-γ) transcription factor [67]. In line with the role of PPAR-γ in fat accumulation, SIRT1 promotes lipolysis and inhibits adipogenesis [67]. The enzyme also binds and deacetylates the MyoD transcription factor and impairs MyoD-driven gene expression and muscle differentiation [19]. The DNA repair protein Ku70 is an additional target of SIRT1. SIRT1-dependent Ku70 deacetylation on Lys539 and Lys542 enables Ku70 to interact with Bax and enables Bax sequestration from mitochondria, thereby preventing stress-dependent apoptosis [68]. These results suggest that SIRT1 is an emerging central regulator of both stress response and apoptotic machinery, with different effects depending on the protein or gene targeted.

Finally, SIRT2 also recognizes non-histone substrates. SIRT2 deacetylates tubulin, possibly regulating microtubule stability, cell structure and motility [23], and interacts with the homeobox transcription factor HOXA10, suggesting a role in mammalian development [69].

Acetyl-ADP-ribose: an orphan metabolite in the midst of metabolic networks

When injected into immature starfish oocytes at concentrations in the range of 0.32–5.00 mM, acetyl-ADP-ribose induces delay and then block of oocyte maturation, whereas at 16 mM acetyl-ADP-ribose quickly triggers oocyte death [32]. The finding that microinjection of human SIRT2 or yeast Hst2 also blocks starfish oocyte maturation and early embryo development [32] suggests that acetyl-ADP-ribose might mediate some of the effects of sirtuins. Evidence that acetyl-ADP-ribose has a short half-life in both oocyte and human cell extracts, together with the ability of ADP-ribose to block oocyte maturation, implies that acetyl-ADP-ribose might affect cell functioning indirectly (Figure 3). Indeed, in human cell extracts acetyl-ADP-ribose can be transformed into ADP-ribose

and acetate by esterases, or function as a co-substrate for unidentified nuclear trans-acetylases. Moreover, purified ADP-ribose hydrolases of the Nudix family metabolize acetyl-ADP-ribose into AMP and acetyl-ribose-5-phosphate, although their intracellular activity remains uncertain [70]. Interestingly, the molecular moiety of acetyl-ADP-ribose resembles that of the intracellular Ca^{2+} receptor agonist cyclic-ADP-ribose. The inability of acetyl-ADP-ribose to prompt intracellular Ca^{2+} release, however, suggests that, at least in oocytes, it is neither a precursor nor a functional analogue of cyclic-ADP-ribose [32]. Thus, data indicate that acetyl-ADP-ribose, directly and/or after conversion to unknown or structurally similar second messengers (Figure 3), operates in as yet unidentified signalling and/or metabolic networks [47].

Therapeutic potential of chemical modulators of sirtuin activity

To date, a significant array of chemical inhibitors and activators of sirtuins have been identified and made available for basic research (Figure 4). Evidence that NAD-dependent deacetylation has a short half-life [11–14] suggests that these chemicals substantially affect sirtuin signalling (Figure 5). Accordingly, the SIRT1 activator resveratrol decreases acetylation-dependent p53 activation and protects human cells from p53-dependent apoptosis [71]. Similarly, resveratrol suppresses Bax-mediated apoptosis probably by boosting SIRT1-induced Ku70–Bax interaction [68]. Resveratrol and other sirtuin-activating compounds (STACs) could also promote FOXO1/3-dependent expression of GADD43, $p27^{kip1}$ and MnSOD, thereby augmenting stress resistance and DNA repair [63–65]. It is conceivable that the ability of resveratrol to hamper p53, Bax and FOXO signalling underlies its pleiotypic health benefits [72].

Alternatively, sirtuin inhibitors might be used to sensitize cells to p53-dependent cell death. Indeed, inhibition of SIRT1 by nicotinamide increases p53 acetylation in mammalian cells exposed to etoposide and the sensitivity of these cells to oxidative stress- or radiation-induced apoptosis [60,61]. Similarly, pharmacological inhibition of SIRT1 relieves its constraint on FOXO3/4, diminishing cellular stress resistance and promoting apoptosis [14,63]. Sirtuin inhibitors, alone or associated to classical DNA-damaging anti-tumor drugs, might therefore be a new class of chemotherapeutics and yield effective cancer therapy. By contrast, the finding that SIRT1 impairs NF-κB transactivation and sensitizes to tumour necrosis factor α (TNF-α)-dependent cell death [66] suggests that inhibition of SIRT1 might also result in cytoprotection. Given the key role of NF-κB in inflammation and neurodegeneration, chemical modulators of sirtuin functioning could emerge as novel immunomodulatory and neuroactive drugs. Consistently, resveratrol promotes, whereas the sirtuin inhibitor sirtinol impairs, SIRT1-induced resistance to axonal degeneration [51]. These findings, together with evidence that SIRT1 protects neurons from apoptosis [63], underscore the key role of the enzyme in neuronal survival and indicate that STACs might be neuroprotective.

The observation that SIRT1 promotes fat mobilization by repressing PPAR-γ indicates that SIRT1-dependent

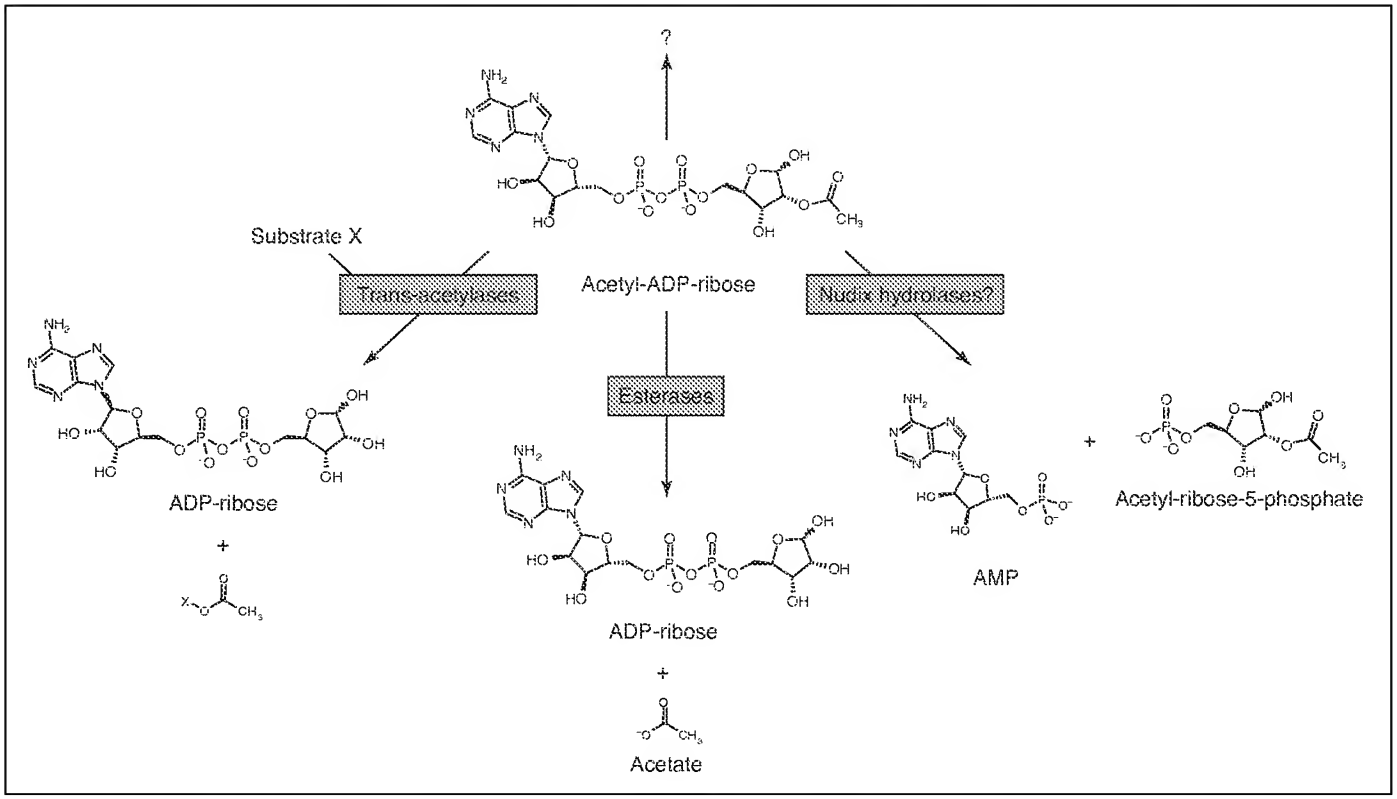

Figure 3. Structure and possible metabolism of acetyl-ADP-ribose. In mammalian cells, acetyl-ADP-ribose can be deacetylated by esterases to the ATP precursor ADP-ribose, or function as an acetyl donor to acetylate unknown substrates (X) by nuclear trans-acetylases. Transformation of acetyl-ADP-ribose into AMP and acetyl-ribose-5-phosphate by ADP-ribose hydrolases of the Nudix family is not clearly established [70]. The possible conversion of acetyl-ADP-ribose into unknown derivatives (?) is shown.

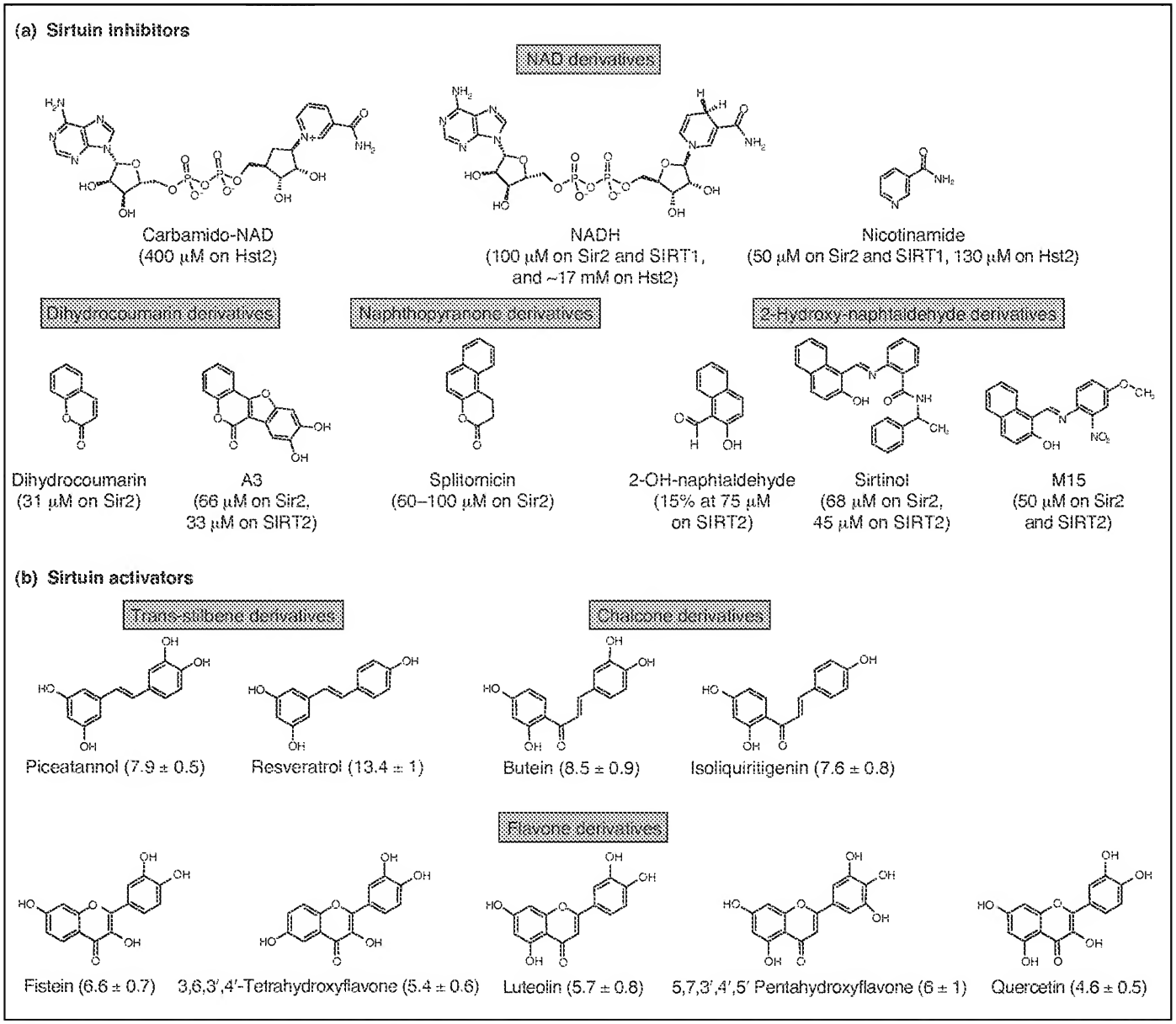

Figure 4. Structures and properties of sirtuin inhibitors and activators. The chemical structures of **(a)** sirtuin inhibitors and **(b)** sirtuin activators are shown. IC_{50} values on different sirtuins for inhibitors and fold-activation of SIRT1 for activators (at 100 μM) are shown in brackets. Among the sirtuin inhibitors, carbamido-NAD, bearing a non-hydrolyzable pyridinium-N-glycosidic bond, competitively inhibits the activity of the Sir2 analogue Hst2 [28]. NADH is a physiological competitive inhibitor of Sir2, SIRT1 and Hst2 that has uncertain activity [39,53], whereas nicotinamide (but not nicotinic acid and its analogues [39]) inhibits Hst2, Sir2 and SIRT1 non-competitively [12,28,38,39]. By means of reverse chemical genetics, sirtinol, A3 and M15 have been shown to inhibit Sir2 and SIRT2. The 2-hydroxy-1-naphtaldehyde moiety of sirtinol also inhibits Sir2 and SIRT2 [77]. Splitomicin targets the acetylated histone-binding site and inhibits Sir2 efficiently and Hst1 partially. The effect of splitomicin on gene expression profiles mimics that of Sir2 or Hst1 deletion, suggesting selectivity [11]. Leu244 on Hst1, corresponding to Tyr298 on Sir2, might underlie the relative resistance of Hst1 to splitomicin. Conversely, 1–2 substitution on splitomicin confers Hst1 selectivity [78], whereas substitutions at the lactone group or in 7–9 are not tolerated [79]. Dihydrocoumarin is a low-toxicity, efficient Sir2 inhibitor, whereas dehydro- and 5-benzyloxy-splitomicin are less potent than splitomicin but selective for Hst1 and Sir2, respectively [79]. Although not shown in the figure, at 100 μM the purine receptor antagonist NF279 (see Chemical names), the G-protein antagonist NF023 and suramin reduce SIRT1 activity by a factor of 285, 625 and 5000, respectively [71]. Among activators, polyphenols such as trans-stilbenes, chalcones and flavones are more powerful activators than isoflavones, flavanones, catechins and anthocyanidins, with the hydroxylated trans-stilbene structure probably conferring the SIRT1 stimulatory activity. Although resveratrol displays the maximal stimulatory activity on SIRT1 (13-fold), compared with the other compounds shown [71], it is less efficient in stimulating Sir2 of yeast, *Caenorhabditis elegans* and *Drosophila* [45]. Resveratrol lowers the K_m of SIRT1 for both the acetylated peptide and NAD (by a factor of 35 and 5, respectively) [71], but the underlying molecular mechanisms remain unknown.

deacetylation is a key regulator of lipid metabolism. Accordingly, resveratrol increases adrenaline-induced fatty acid release from adipocytes, an event that is suppressed by nicotinamide [67]. Drugs that regulate sirtuin activity, therefore, might be of therapeutic significance in metabolic disorders, insulin resistance and diabetes. SIRT1-dependent inhibition of adipogenesis might also have a role in atherosclerosis, and STACs could emerge as innovative drugs to treat or prevent cardiovascular disorders. In keeping with this, SIRT1 protects cardiomyocytes from p53-dependent apoptosis and its expression is increased during heart failure [73]. Finally, given the ability of SIRT1 to block muscle differentiation, chemical regulators of its deacetylating

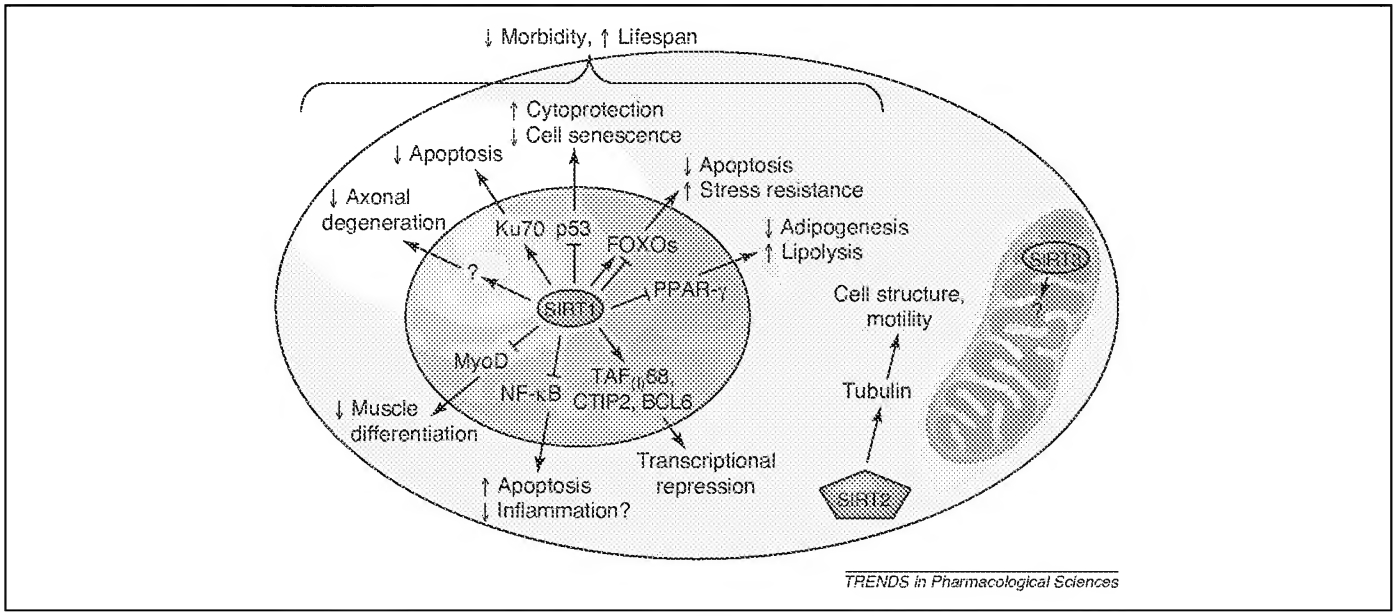

Figure 5. Cellular targets of mammalian sirtuins. Nuclear SIRT1 targets several chromatin-interacting proteins with key roles in transcription, stress response and death. Regulation of these proteins by SIRT1-dependent deacetylation might result in enhanced cellular resistance to stress, reduced morbidity and lifespan extension. SIRT2 is mainly cytoplasmic and deacetylates tubulin, suggesting a role in microtubule organization in addition to cell structure and motility. SIRT3 is a mitochondrial deacetylase with unknown functions. Pharmacological modulation of sirtuin activity by enzymatic activators or inhibitors can therefore significantly affect cellular functioning and be harnessed to therapeutic interventions (see main text for further details). Abbreviations: PPAR-γ, peroxisome proliferator-activated receptor γ.

activity such as nicotinamide [19] might be harnessed to promote muscle regeneration and have a role in the treatment of muscular diseases.

As a result of data obtained primarily from studies in yeast, the hypothesis that STACs increase longevity in mammals has been proposed. Of course, claiming that mechanisms that underlie human aging are identical to those operating in yeast is oversimplified. Nevertheless, the fact that SIRT1 activation is a prerequisite for both resveratrol and CR to extend lifespan in multicellular organisms [45], together with evidence that CR has beneficial effects on age-associated morbidity in primates [74], suggests that sirtuin activity is of relevance to human aging. Yet, one might argue that impairment of p53 surveillance by STACs might promote neoplastic transformation and ultimately decrease life expectancy. Although this is an obvious potential pitfall of drugs that activate SIRT1, evidence that increased p53 activity accelerates ageing [75] suggests that keeping p53 in check with STACs can prolong longevity. The hypothesis that p53 impairment by SIRT1 promotes neoplastic transformation is also challenged by data showing that SIRT1 activators such as CR and resveratrol reduce tumour incidence in mammals [43,72]. Notably, if it is confirmed that sirtuin-dependent deacetylation concurs to determine the pace of human aging, then STACs will be the first class of drugs that interact directly with a pervasive mechanism through which genetic machinery regulates senescence in humans. Finally, it will be important to determine whether class I HDACs, class II HDACs and sirtuins share common targets, and deacetylation by the different classes of HDACs is somehow redundant. Evidence that maximal p53 and Ku70 acetylation occurs when class I HDACs, class II HDACs and sirtuins are inhibited in tandem suggests that sirtuin-dependent deacetylation cannot be compensated by the other HDACs [60,76]. These findings emphasize the pharmacological significance of sirtuin-interacting drugs, and also suggest that identification of substrates specifically targeted by a single class of HDACs would have considerable therapeutic implications.

In conclusion, sirtuin deacetylases emerge as unique enzymes involved in epigenetics, cell death and lifespan regulation (Figure 5). Pharmacological interventions aimed at regulating sirtuin activity have proved successful, and it is therefore conceivable that in the near future current leads will be developed into more-selective and powerful drugs of therapeutic relevance to human disorders.

Chemical names

NF023: 8,8′-[carbonylbis(imino-3,1-phenylene)] bis-(1,3,5-naphtalenetrisulfonic acid)
NF279: 8,8′-[carbonylbis(imino-4,1-phenylene)] bis-(1,3,5-naphtalenetrisulfonic acid)

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